Electronic Supplementary Information

Part 1. Experimental

Chemicals

Graphite powder, Urea and Ammonium bicarbonate (NH_4HCO_3), Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Ethylene glycol (EG), Diethylene glycol (DEG), Ethylenediamine (EN) and Sodium acetate (NaAc) were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). Lanthanum nitrate hexahydrate ($La(NO_3)_3 \cdot 6H_2O$), Trifluoroacetic acid (TFA), Sodium phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), (3-Mercaptopropyl)trimethoxysilane (98%, MPS), Acetonitrile (ACN), Ammonium bicarbonate (NH_4HCO_3), 2, 5-Dihydroxybenzoicacid (DHB), Dithiothreitol (DTT), Bovine serum albumin (BSA) and Commercial TiO_2 nanoparticles were purchased from Aladdin (Shanghai, China). Bovine β-casein and Trypsin (from bovine pancreas, TPCK treated) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iodoaceticacid (IAA) was purchased from Alfa Aesar (USA). $K_2S_2O_8$, P_2O_5, $KMnO_4$, H_2O_2 (30%), H_2SO_4, HCl and Ethanol were all analytical grade reagents and were obtained from Beijing Chemical Regent Co. Ltd. (Beijing, China). Commercial Zip-TipC18 pipette tips were purchased from Millipore (USA). Human urine and serum samples were offered by a local hospital from a healthy volunteer. All the chemical agents were used without further purification.

Preparation of superparamagnetic Fe_3O_4 particles

For synthesis of the Fe_3O_4 nanoparticles, $FeCl_3 \cdot 6H_2O$ (2.50 g) was added into a mixing solution containing 8.9 mL of EN and 80 mL of EG under magnetic stirring, followed by the addition of NaAc (5.00 g). The obtained mixture was transferred into a Teflon-lined stainless-steel autoclave and sealed to heat at 200℃ for 6 h. The produced magnetite nanoparticles were washed with deionized water, separated using a magnet, and finally dried in an oven at 80 ℃ for 12 h.

Preparation of GO

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method.[1]

Preparation of LaG and $LaPO_4$ nanorods

For preparation of lanthanum phosphate graphene oxide composites (LaG), 50 mL of the as-synthesized GO with a concentration of approximately 1 g L^{-1} was further dispersed via ultrasonication for 30 min to form a GO suspension. 92.5 mg of $La(NO_3)_3 \cdot 6H_2O$ was dissolved in 50 mL of deionized water, and the solution was slowly injected into GO dispersion system under magnetic stirring and further stirred for 3 h.

Then, 25 mg of $NaH_2PO_4 \cdot 2H_2O$ dissolved in 10 mL of deionized water and slowly added into the above suspension. The resulting solution was stirred at room temperature for 12 h. The LaG product was obtained after separated by centrifugation, washed with deionized water for three times and lyophilized to dryness.

Synthesis of LaGM

To conjugate the LaG with MPS, 25 mg of LaG was redispersed into 50 mL of ethanol via ultrasonication for 1h at room temperature. The suspension was then bubbled with nitrogen for 30 min under mechanical agitation. Subsequently, 0.5 mL of MPS was slowly injected into the dispersion system under nitrogen atmosphere followed mechanically stirring for 6 h at 70℃ in constant water bath. The MPS-modified LaG products were obtained after separated by centrifugation and washed with ethanol for several times to remove the unreacted MPS.

The MPS modified LaG and the as-synthesized Fe_3O_4 nanoparticles were respectively re-dispersed into 25 mL ethanol via ultrasonication for 30 min to form two suspension solutions. Then, the Fe_3O_4 particle suspension was added dropwise into the MPS-modified LaG suspension solution under agitation. The mixture was further stirred for 12 h at room temperature. The precipitate was collected by a magnet, and then washed with water for several times, and subsequently re-dispersed into 50 mL of water via ultrasonication for 30 min. The pH value of the above dispersion solution was adjusted to 8.0 using dilute ammonia solution. Subsequently, 1.0 g urea was added into the mixture solution under agitation, and then the mixture solution was heated to 95℃. After stirring for 30 h, the final products (LaGM) were collected by magnetic separation, and washed with deionized water for several times.

Tryptic digest of proteins

1 mg protein (β-casein or BSA) was dissolved in 1 mL of 50 mM NH_4HCO_3 solution, and then trypsin was added into the solution with a molar ratio of 50:1 (substrate/trypsin) for 18 h at 37℃. Finally, the obtained tryptic digests were diluted to the target concentration.

Enrichment of low-abundance peptides and Capture of phosphopeptides from the complex sample

Enrichment of low-abundance peptides. 1000 μL (10 nM) BSA digest was mixed with 10 μL of 35 mg/mL LaGM composites suspending solution and then shaken for 15 min. Subsequently, the composites trapped target peptides were collected by magnetic separation. Then, the enriched peptides were eluted with 5 μL of elution solution containing 60% ACN and 1.0% TFA, and the supernatant was collected by magnetic separation. For enriching peptides from the human urine, 1000 μL urine was mixed with 10 μL of 35 mg/mL LaGM composite suspending solution and then shaken for 15 min. Subsequently, the

composites trapped target peptides were collected by magnetic separation and washed with 500 μL of water for two times. Then, the enriched peptides were eluted with 5 μL of elution solution containing 60% ACN and 1.0% TFA, and the supernatant was collected by magnetic separation.

Capture of phosphopeptides. 50 μL (100 nM) β-casein digest was mixed with 10 μL of 35 mg/mL LaGM composite suspending solution and then shaken for 5 min. Subsequently, the composites trapped target peptides were collected by magnetic separation. To purify the obtained phosphopeptides, the particles were washed with 80 μL acetonitrile/deionized water (1/1, v/v) solution containing 0.5% TFA for twice. Samples of β-casein digests of 5×10^{-9} M, 1×10^{-9} M, and 5×10^{-10} M were used to evaluate the sensitivity of the LaGM affinity probe. For the peptide mixture of β-casein and bovine serum albumin (BSA) (1:10, molar ratio), three buffers with different concentrations of TFA (v/v; 0.5%, 1.0% and 1.5%) were used to optimize the affinity selectivity. After that, the trapped phosphopeptides were eluted with 30 μL of 3% ammonium hydroxide, and the supernatant was collected by magnetic separation and lyophilized to dryness. Fe_3O_4 nanoparticles were also investigated for comparison, and the procedure for capture of phosphopeptide is similar to LaGM.

Capture of the low-abundance peptides and phosphopeptides from human serum

20 μL of pristine serum was diluted with 200 μL of water to form a serum solution. 10 μL of 35 mg/mL LaGM composite suspending solution was added into the serum solution and then shaken for 15 min. The composites enriched target peptides were collected by magnetic separation. Then, the enriched peptides were eluted with 5 μL of elution solution containing 60% ACN and 1.0% TFA, and the supernatant was collected by magnetic separation. Subsequently, the LaGM composites were further washed with 80 μL acetonitrile/deionized water (1/1, v/v) solution containing 0.5% TFA for two times. After that, the trapped phosphopeptides were eluted with 30 μL of 3% ammonium hydroxide, and the supernatant was collected by magnetic separation and lyophilized to dryness. For comparison, commercial ZipTipC18 pipette tip and TiO_2 nanoparticles were also used to capture target peptides from diluted human serum using the similar procedure.

MALDI-TOF MS analysis and Search parameters

The above products obtained from the elution step were dissolved in 50% (v/v) aqueous acetonitrile solution (2 μL) containing DHB (20 mg/mL) and H_3PO_4 (1%, v/v) by pipetting, and the mixture was then deposited onto the MALDI target. MS analysis was carried out on a Bruker Autoflex III MALDI-TOF MS (Bruker Daltonics Bremen, Germany) equipped with a 355 nm Nd-YAG laser. The acceleration voltage

and repetition rate were set at 19 kV and 200 Hz, respectively, whilst the laser power was optimized until the best-quality spectra was obtained. For peptide mass fingerprinting (PMF) data, 600 laser shots were accumulated for each spectrum, which was obtained in positive ion reflection mode and analyzed by Bruker Daltonics flex Analysis software. Search parameters fragment ion spectra were submitted to MASCOT (http://www.matrixscience.com/) for database search and identification of corresponding peptides employing the following search parameter settings. Database: NCBInr; Enzyme: Trypsin; Maximum of missed cleavages: 1; Taxonomy: Mammalia (mammals); Peptide tolerance: 1.2 Da; Mass values: Monoisotopic.

General Techniques

Scanning electron microscopy (SEM) images were performed on a field emission scanning electron microscope (FESEM, S4800, Hitachi) equipped with an energy-dispersive X-ray analysis system (EDXA, JEOLJXA-840). Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were taken with a FEI Tecnai G2 S-Twin transmission electron microscope operated at 200 kV. Powder X-ray diffraction (XRD) patterns were collected on a Bruker D8FOCUS X-ray diffractometer using Cu Kα radiation (λ=1.5406 Å) at a scanning rate of 15^{o} min^{-1} and the detective range from 10-80°. Fourier-transform infrared spectroscopy (FT-IR) analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. Magnetization measurement was carried out with a superconducting quantum interface device (SQUID) magnetometer (Quantum Design MPMS XL) at 300 K.

Part 2. Supporting figures and tables

Supporting figures

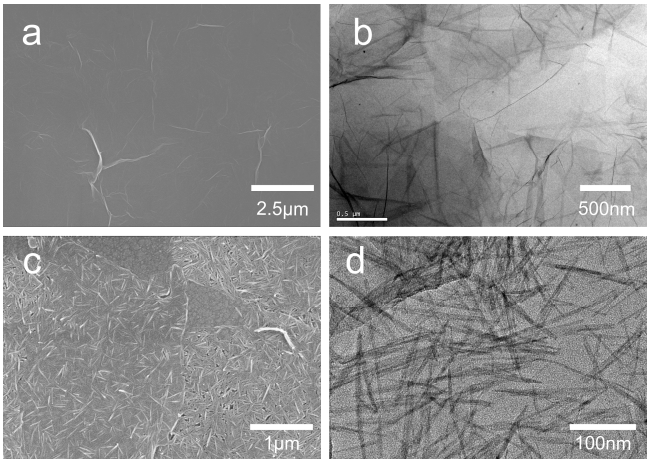

Fig. S1 SEM and TEM images of (a, b) GO and (c, d) LaG.

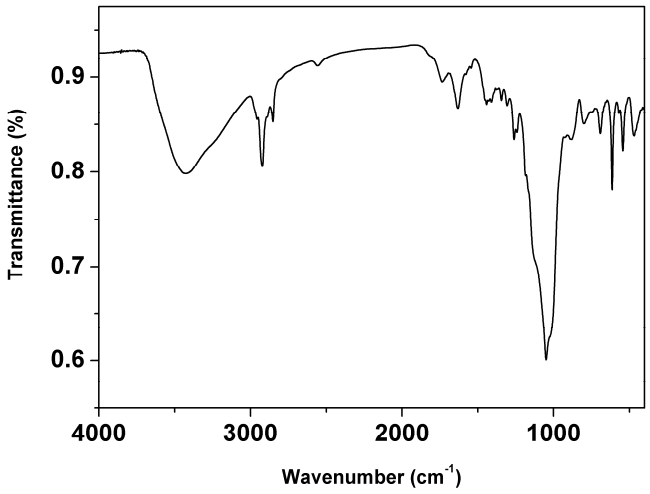

Fig. S2 The FTIR specturm of the MPS-modified LaG.

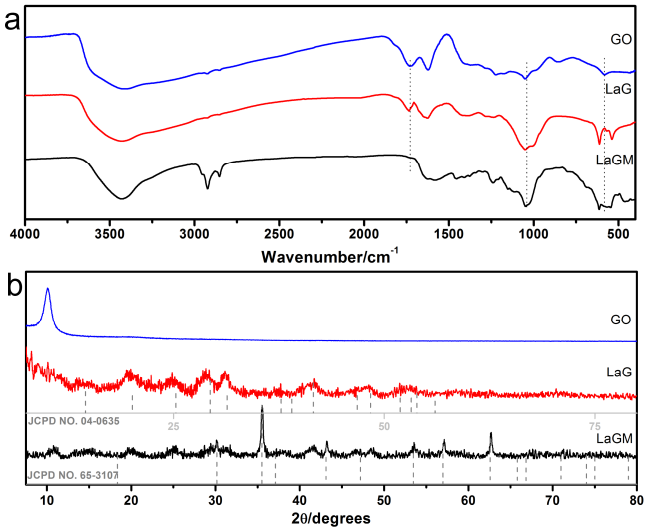

Fig. S3 (a) FTIR spectra and (b) XRD patterns of GO, LaG and LaGM.

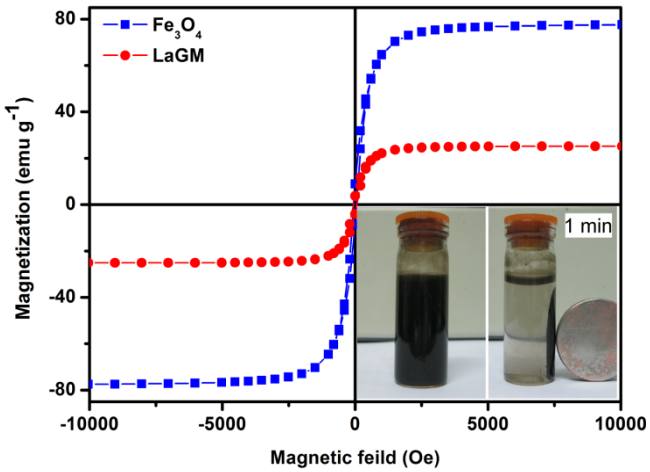

Fig. S4 Hysteresis loops of Fe_3O_4 nanoparticles and LaGM composites at 300K and the dispersion and the separation photographs of LaGM composites.

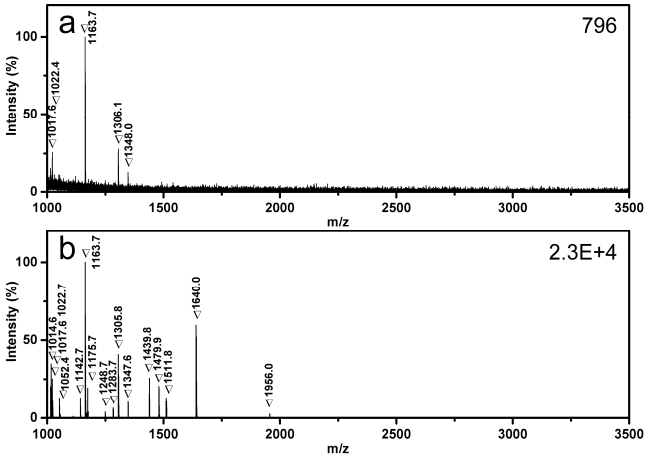

Fig. S5 MALDI-TOF mass spectra of (a) 10 nM BSA digest without any treatment, (b) 10 nM BSA digest after enrichment by LaGM affinity probe. The number in the top right corner is the highest peak intensity.

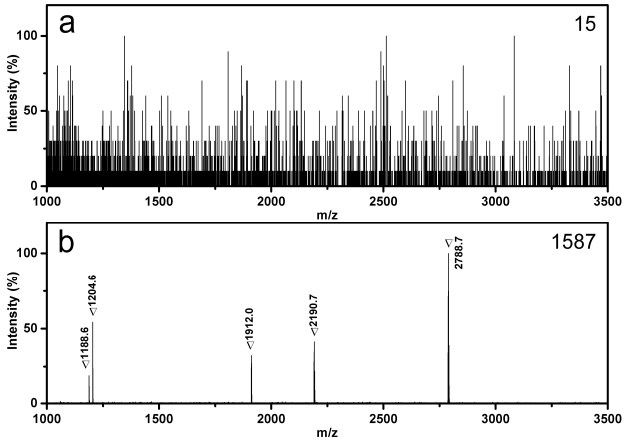

Fig. S6 MALDI-TOF mass spectra of (a) human urine without any treatment, (b) human urine after enrichment by the LaGM affinity probe. The number in the top right corner is the highest peak intensity.

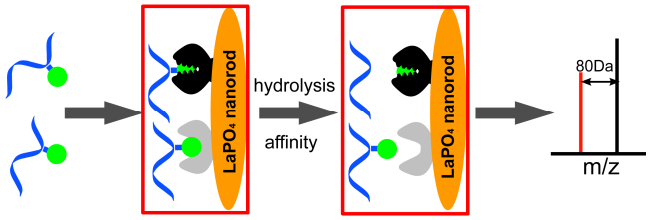

Fig. S7 Schematic illustration of the capturing and labeling mechanism of the $LaPO_4$ nanorods on LaGM.

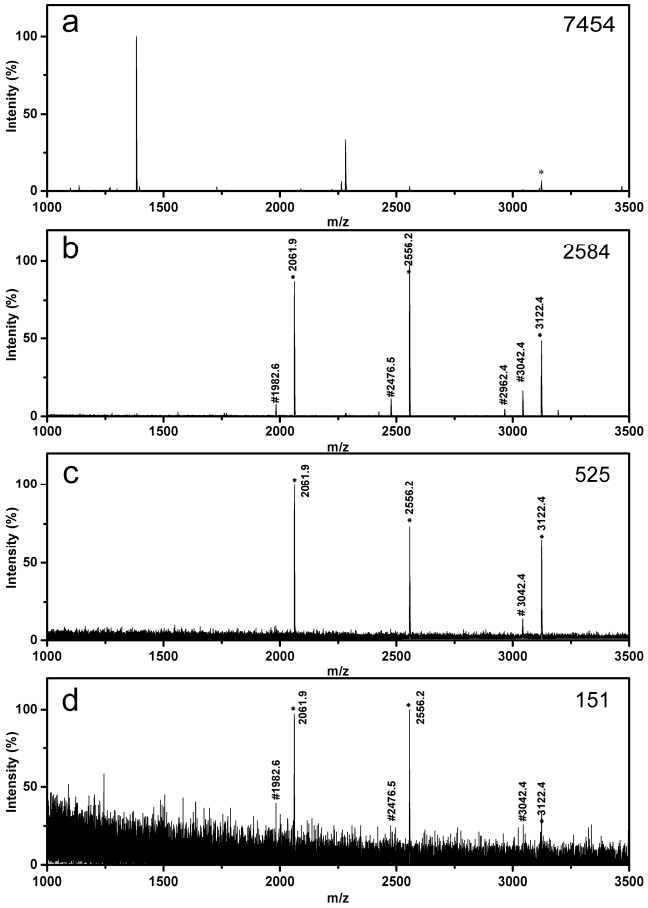

Fig. S8 MALDI-TOF mass spectra of (a) β-casein digest without any treatment, (b) β-casein digest after captured by LaGM affinity probe, (c) β-casein digest after captured by pure Fe_3O_4 nanoparticles, and (d) highly diluted β-casein digest (10^{-9} M) after capture by LaGM affinity probe. The number in the top right corner is the highest peak intensity.

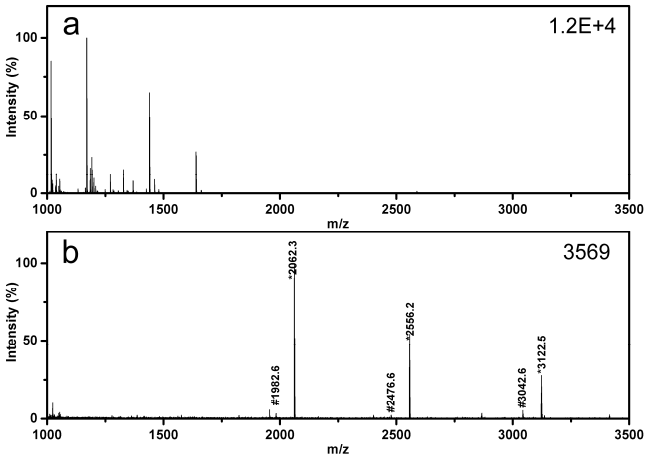

Fig. S9 MALDI-TOF mass spectra of (a) the digest mixtures of β-casein and BSA (1:50; molar ratio) without any treatment, and (b) the digest mixtures of β-casein and BSA after capture by LaGM affinity probe. The number in the top right corner is the highest peak intensity.

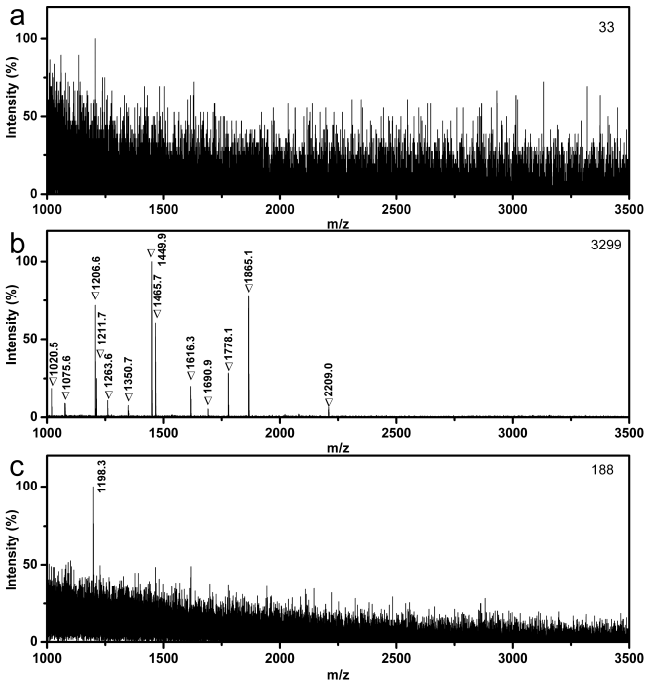

Fig. S10 The MALDI-TOF mass spectra of the diluted human serum solution a) without any treatment, b) the low-abundance peptides enriched by commercial ZipTipC18 pipette tip in the first step, and c) the peptides captured by commercial ZipTipC18 pipette tip in the second step. The numbers in the corner are the highest peak intensities. Although thirteen peptides can be detected in the first elution step, no phosphopeptides can be detected in the second elution step.

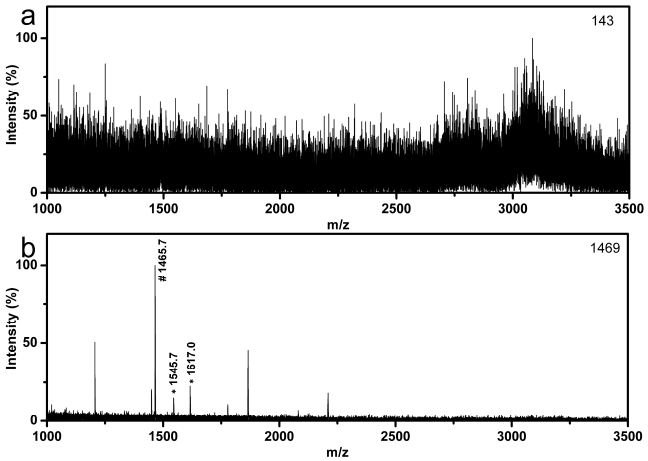

Fig. S11 The MALDI-TOF mass spectra of the diluted human serum solution a) the low-abundance peptides enriched by commercial TiO_2 nanoparticles in the first step, and b) the peptides captured by commercial TiO_2 nanoparticles in the second step. The numbers in the corner are the highest peak intensities. Although the TiO_2 affinity particles can extract the phosphopeptides from the sample, they are disturbed by some nonphosphopeptides and most of the useful and informative low-abundance peptides are lost during first enrichment step and no longer contribute to the practical application.

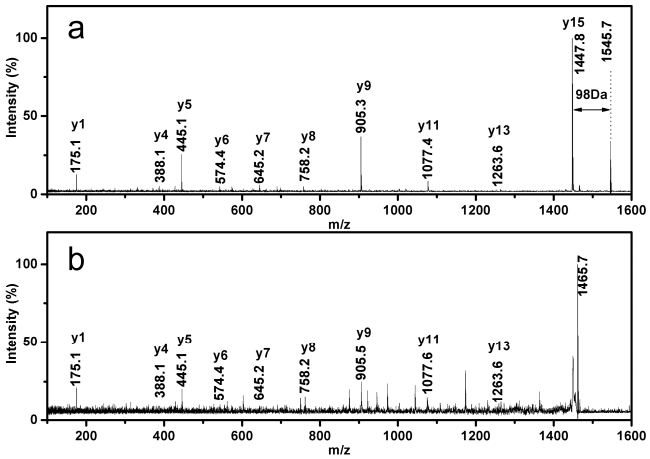

Fig. S12 MALDI-TOF/TOF mass spectra of human serum for the precursor ion at a) m/z 1545.7, and b) the corresponding label ion at m/z 1465.7. The peptide at m/z 1545.7 is phosphopeptide due to the presence of the fragment ion adjacent to the parent ion with a mass difference of 98 Da in MS/MS spectrum. While the peptide at m/z 1465.7 without phosphorylation is the label of the phosphopeptide at m/z 1545.7, because it has the same sequence as the precursor ion.

Supporting tables

Table S1. The search results of the tryptic digest of BSA before and after enrichment by the LaGM affinity probes

AA	Calculated (m/z)	Peptide sequences	Before enrichment	After enrichment
286-294	1014.48	SHCIAEVEK		√
188-196	1016.57	VLTSSARQR	√	√
475-483	1023.45	CCTESLVNR	√	√
436-444	1051.44	CCTKPESER		√
524-533	1141.71	KQTALVELLK		√
42-51	1162.62	LVNELTEFAK	√	√
276-285	1176.55	ECCDKPLLEK		√
11-20	1248.61	FKDLGEEHFK		√
337-347	1282.70	HPEYAVSVLLR		√
378-388	1304.71	HLVDEPQNLIK	√	√
52-64	1348.54	TCVADESHAGCEK	√	√
336-347	1439.81	RHPEYAVSVLLR		√
397-409	1478.79	LGEYGFQNALIVR		√
414-427	1510.44	VPQVSTPTLVEVSR		√
413-427	1638.93	KVPQVSTPTLVEVSR		√
295-312	1954.95	DAIPENLPPLTADFAEDK		√
Peptides matched			5	16
Sequence coverage (%)			7	27

Table S2. The phosphopeptides and their labeling signals identified by MALDI-TOF MS from tryptic digest of β-casein.

AA	Peptide sequences	Observed *m/z*	Theoretical *m/z*	Phosphorylation site
33-48	FQ[pS]EEQQQTEDELQDK	2061.8	2061.8	1
33-52	FQ[pS]EEQQQTEDELQDKIHPF	2556.1	2556.0	1
1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3122.3	3122.2	4

Reference

1. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339-1339; D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu and J. M. Tour, *ACS Nano*, 2010, **4**, 4806-4814.